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(54) Title: COMPOSITIONS OF ORTHOGONAL GLUTAMYL-TRNA AND AMINOACYL TRNA SYNTHETASE PAIRS AND USES THEREOF

(57) Abstract: Compositions and methods of producing components of protein biosynthetic machinery that include glutamyl orthogonal tRNAs, glutamyl orthogonal aminoacyl-tRNA synthetases, and orthogonal pairs of glutamyl tRNAs/synthetases are provided. Methods for identifying these orthogonal pairs are also provided along with methods of producing proteins using these orthogonal pairs.

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(57) Abstract: Compositions and methods of producing components of protein biosynthetic machinery that include glutamyl orthogonal tRNAs, glutamyl orthogonal aminoacyl-tRNA synthetases, and orthogonal pairs of glutamyl tRNAs/synthetases are provided. Methods for identifying these orthogonal pairs are also provided along with methods of producing proteins using these orthogonal pairs.

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# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US04/21813

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : C12N 9/10, 15/12, 15/54; C12P 21/00
US CL : 435/252.3, 193, 91.4,320.1, 13, 69.1; 536/23.2
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 435/252.3, 193, 91.4,320.1, 13, 69.1; 536/23.2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| P, X | Santoro WS et al. An archaebacteria-derived glutamyl-tRNA synthetase and tRNA pair for unnatural amino acid mutagenesis of proteins in E. coli. October 12, 2003. Vol 31, No. 23, pages 6700-6709. | 1-49 |
| A | Liu RD et al. Characterization of an orthogonal suppressor tRNA derived from E. coli glutaminyl-tRNA. Chemistry and Biology. August 1997, Vol. 4 No. 9, pages 685-691. | 1-49 |
| A | Kowal et al Twenty-first amino-acyltRNA synthetase-suppressor tRNApairs for possible use in site-specific incorporation of amino acid analogues into proteins in eukaryotes and in eubacteria. PNAS, February 2001, Vol. 98, No. 5 pages 2268-2273. | 1-49 |

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
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Date of mailing of the international search report
05 JAN 2006

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# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US04/21813

**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☒ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of any additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US04/21813

## BOX III. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I- Claims 1-13, 15-23 are drawn to a composition comprising an orthogonal glutamyl-tRNA (O-tRNA$^{glu}$) and an orthogonal tRNA synthetase (glutamyl-O-RS) and an amber selector codon.

Group II- Claims 1-11, 14 are drawn to a composition comprising a translation system wherein the translation system comprises an orthogonal glutamyl-tRNA (O-tRNA$^{glu}$) and an orthogonal tRNA synthetase (glutamyl-O-RS) and a selector codon.

Group III- Claims 24-28 is drawn to an artificial polynucleotide of SEQ ID NO: 67.

Group IV- Claims 29-36 are drawn to a method of producing a pool of orthogonal tRNA (O-tRNA$^{glu}$ ) to a selected cell.
Group V- Claims (37-39, 42, 43 in part) and 41 are drawn to a method for identifying an O-tRNA synthase for use with an O-tRNA$^{glu}$ in a cell.

Group VI - Claims (37-39, 42, 43 in part) and 40 are drawn to a method for identifying an O-tRNA synthase for use with an O-tRNA$^{glu}$ in vitro.

Group VII - Claims 44-49 are drawn to a method of producing a protein in a cell with a selected amino acid at specific in a cell with a selected amino acid at specified position.

The inventions listed as Groups I-VII do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The technical feature linking the inventions in group I-VII is a method of producing a composition of orthogonal glutamyl-tRNA and orthogonal tRNA synthetase pairs used in a method of producing proteins comprising unnatural amino-acid(s). However Santoro et al disclose an archaebacteria-derived glutamyl-tRNA synthetase and tRNA pair for unnatural amino acid mutagenesis of proteins in E. coli wherein the amino acid specificity of an orthogonal tRNA synthetase is is modified to charge the corresponding orthogonal tRNA with an unnatural amino acid that is subsequently incorporated into a polypeptide in response to a nonsense or missense codon. Thus the technical feature linking the invention of Groups I-VII is not a special technical feature as defined by PCT Rule 13.1, because under Rule 13.2, it does not contribute a special technical feature over the prior art.